Role of the brain iso-renin–angiotensin system in experimental hypertension in rats

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Summary

1. To examine the possible participation of the brain iso-renin–angiotensin system in the control of blood pressure, as well as in the regulation of plasma renin activity, saralasin and captopril were injected into the cerebral ventricles of three types of experimental hypertensive rats with different plasma renin profiles.

2. Injection of saralasin and captopril into the cerebral ventricles resulted in a significant decrease in blood pressure of two-kidney, one-clip Goldblatt hypertensive rats (11 ± 2 and 9 ± 3 mmHg respectively) and that of spontaneously hypertensive rats (13 ± 2 and 12 ± 2 mmHg respectively), but in deoxycorticosterone (DOC)–salt hypertensive rats injection of these two agents showed a significant increase in blood pressure (13 ± 2 and 12 ± 3 mmHg respectively).

3. The plasma renin activity was markedly decreased after injection of saralasin and captopril into the cerebral ventricles of two-kidney, one-clip Goldblatt hypertensive rats. Conversely, in DOC–salt hypertensive rats, the plasma renin activity was markedly increased after injection of these two agents. In spontaneously hypertensive rats these agents caused no significant change in plasma renin activity.

4. These findings suggest that the brain iso-renin–angiotensin system participates in the central regulation of blood pressure and may be responsible for modulation of the peripheral renin–angiotensin system.

Key words: angiotensin II, brain, captopril, hypertension, iso-renin–angiotensin system, plasma renin activity, saralasin, vasopressin.

Abbreviation: DOC, deoxycorticosterone.

Introduction

Since iso-renin (or renin), angiotensinogen, angiotensin I, angiotensin II and angiotensin-converting enzyme were reported to be present in the brain or in the cerebrospinal fluid in a number of mammalian species [1], considerable attention has been focused on the role of the brain iso-renin–angiotensin system in the central regulation of blood pressure [2–4]. Intraventricular administration of angiotensin II caused an increase in blood pressure in normotensive rats [5, 6]. Furthermore the angiotensin II antagonist, saralasin, decreased the blood pressure in rats with renal hypertension [7], in spontaneously hypertensive rats [8] and in malignant hypertensive rats [9].

Captopril, a converting enzyme inhibitor, has been widely used to evaluate the contribution of the renin–angiotensin system in the maintenance of high blood pressure. Since these agents have been reported not to cross the blood–brain barrier [10, 11], we examined the effects of injection of captopril and of saralasin into the cerebral ventricles on blood pressure in three different types of hypertension. We also studied the changes in peripheral plasma renin activity after intraventricular injection of saralasin or captopril. For the three types of experimental hypertension, two-kidney, one-clip Goldblatt hypertensive rats with high renin, DOC–salt hyper-
tensive rats with low renin and spontaneously hypertensive rats with normal renin were used.

Materials and methods

Animals

Male Wistar rats and male spontaneously hypertensive rats and their respective normotensive Wistar–Kyoto controls were used in the study. The rats were kept in Nacrolon cages in an automatically lit room with constant temperature and humidity. The standard diet contained 100 mmol of sodium and 210 mmol of potassium/kg. Tap water was offered ad libitum to the spontaneously hypertensive rats, Wistar-Kyoto control rats, two-kidney, one-clip Goldblatt hypertensive rats and their sham-operated control rats. DOC-salt hypertensive rats and their sham-operated control rats received 1% sodium chloride solution (171 mmol/l: 1% saline).

Experimental groups

Male Wistar rats were used in groups 1–3. Operation for induction of hypertension was performed under ether anaesthesia. Rats of groups 1 and 2 were tested 20–22 days after the operation.

Groups 1A and B. Rats weighing 140–160 g were used. A: two-kidney, one-clip Goldblatt hypertension. The left renal artery was constricted with a silver clip (0.2 mm internal diameter) leaving the contralateral kidney untouched (n = 24). B: sham-operated control rats. The left renal artery was exposed through a dorsal midline incision and a silver clip was left in the perinephric fat (n = 18).

Groups 2A and B. Rats weighing 100–120 g were used. A: DOC-salt hypertension. After nephrectomy, the rats were treated daily with deoxycorticosterone trimethylacetate (DOC-TMA) (Percorten M; Ciba, Basel) at a dose of 1 mg/kg, intramuscularly (n = 24). B: after nephrectomy, the rats were treated with sesame oil (n = 18).

Groups 3A and B. A: spontaneously hypertensive rats (20 weeks old, weighing 180–200 g; n = 24) and B: their respective age-matched Wistar–Kyoto control rats (n = 18).

Blood pressure monitoring

In all rats a 0·11 mm polythene cannula was placed via the femoral artery into the abdominal aorta, 0·5–1·0 cm proximal to the aortic bifurcation, led subcutaneously out through an incision in the back and filled with heparin/0·9% sodium chloride solution (145 mmol/l: 0·9% saline). To record blood pressure, the cannula was connected to a pressure transducer (Nippon Koden RM-25 recorder). The insertion of the femoral arterial catheter was performed under ether anaesthesia 2 days before the experiment. Elevation of blood pressure in the experimental hypertensive rats was confirmed before the test (Table 1).

Intraventricular injections

A 21-gauge guide cannula was implanted at the bregma and 1·3 mm lateral of the midline and fixed to the skull with dental cement. The guide cannula penetrated 4 mm below the surface of the skull and a 27-gauge stylus kept the cannula patent. The implantation of the cerebroventricular catheter was performed under ether anaesthesia 24 h before the experiment. Blood pressure was recorded continuously in the conscious rats, before, during and after the intraventricular injection of the agents. Both saralasin (Protein Research Foundation, Osaka, Japan) and captopril (SQ 14 225, E. R. Squibb and Sons

| Table 1. Body weight, systolic blood pressure and plasma renin activity on the test day |
|----------------------------------------|-----------------|------------------|
| Rats                                   | Body weight (g) | Systolic blood pressure (mmHg) | Plasma renin activity (pmol of ANG I h⁻¹ ml⁻¹) |
| Two-kidney, one-clip Goldblatt hypertensive (n = 8) | 280 ± 16        | 180 ± 6*          | 7·2 ± 0·5*       |
| Sham-operated control (n = 6)           | 275 ± 12        | 118 ± 4*          | 3·6 ± 0·6*       |
| DOC-salt hypertensive (n = 8)            | 253 ± 22        | 164 ± 8*          | 1·2 ± 0·3*       |
| Sham-operated control (n = 6)           | 241 ± 6         | 110 ± 6*          | 3·2 ± 0·4*       |
| Spontaneously hypertensive (n = 8)       | 196 ± 12        | 178 ± 8*          | 3·6 ± 0·5       |
| Wistar–Kyoto control (n = 6)             | 212 ± 18        | 102 ± 6*          | 3·4 ± 0·3       |

Values are means ± SEM. Significance of differences from controls: *P < 0·01. n = Number of animals.
Inc., Princeton, NJ, U.S.A.) were diluted in 0.9% saline. Saralasin was injected intraventricularly at a dose of 4 μg/min for 5 min and captopril at a dose of 10 μg/10 μl; 10 μl of 0.9% saline was given as a control.

Saralasin or captopril was injected into the cerebral ventricle of different animals. These injections were done in the same number of rats in each experimental group.

Blood samples were obtained through the catheter implanted in the femoral artery half-an-hour after the intraventricular injection of saralasin, captopril and 0.9% saline.

Plasma renin activity was measured by the method of Skinner [12]. Control values of the plasma renin activity were obtained 6 h before the experiment (Table 1).

Values are given as means ± SEM. For statistical analysis, Student’s t-test was used.

Results

Blood pressure changes after intraventricular injection of saralasin and captopril (Fig. 1)

In sham-operated rats and Wistar-Kyoto control rats intraventricular injection of saralasin and captopril failed to cause any significant change in blood pressure. In two-kidney, one-clip Goldblatt hypertensive rats and spontaneously hypertensive rats intraventricular injection of saralasin and captopril produced a significant decrease in blood pressure ($P < 0.01$) (Fig. 2). Conversely, in DOC–salt hypertensive rats and in rats given 1% saline, saralasin and captopril caused a significant increase in blood pressure ($P < 0.01$).

Effects of intraventricular injection of saralasin and captopril on plasma renin activity (Table 2)

In Wistar–Kyoto and sham-operated control rats intraventricular injection of saralasin and captopril caused no significant changes in plasma renin activity. When saralasin and captopril were given intraventricularly to two-kidney, one-clip Goldblatt hypertensive rats, there were significant decreases in plasma renin activity ($P < 0.01$). In DOC–salt hypertensive rats suppressed plasma renin activity was significantly stimulated by saralasin and captopril ($P < 0.01$). Similar changes in plasma renin activity were also observed in rats given 1% saline ($P < 0.05$). In spontaneously hypertensive rats intraventricular injection of the two agents caused no significant changes in plasma renin activity.
FIG. 2. Trace of changes of blood pressure after intraventricular injection of captopril in spontaneously hypertensive rats.

**TABLE 2. Changes of plasma renin activity (pmol of ANG I h⁻¹ ml⁻¹) after injection of saralasin and captopril**

Values are means ± SEM. Significance of differences from controls: *P < 0.01; **P < 0.05. n = Number of animals.

<table>
<thead>
<tr>
<th>Rats</th>
<th>1% saline</th>
<th>Saralasin</th>
<th>Captopril</th>
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<tbody>
<tr>
<td>Two-kidney, one-clip</td>
<td></td>
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<tr>
<td>Goldblatt hypertensive (n = 8)</td>
<td>6.0 ± 1.0*</td>
<td>3.6 ± 1.3*</td>
<td>4.0 ± 0.9*</td>
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<tr>
<td>Sham-operated control (n = 6)</td>
<td>3.5 ± 0.3</td>
<td>3.9 ± 0.5</td>
<td>4.0 ± 0.4</td>
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<tr>
<td>DOC-salt hypertensive (n = 8)</td>
<td>1.1 ± 0.2*</td>
<td>3.0 ± 0.3*</td>
<td>4.3 ± 0.4*</td>
</tr>
<tr>
<td>Sham-operated control (n = 6)</td>
<td>1.5 ± 0.3**</td>
<td>2.5 ± 0.5***</td>
<td>2.7 ± 0.7**</td>
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<tr>
<td>Spontaneously hypertensive (n = 8)</td>
<td>3.8 ± 0.2</td>
<td>3.7 ± 0.4</td>
<td>3.6 ± 0.3</td>
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<tr>
<td>Wistar-Kyoto control (n = 6)</td>
<td>3.5 ± 0.3</td>
<td>3.8 ± 0.6</td>
<td>3.8 ± 0.6</td>
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**Discussion**

In the present study the role of the brain iso-renin–angiotensin system in the central regulation of blood pressure was investigated by intraventricular injection of saralasin and captopril in three types of experimental hypertension.

Although there is a possibility that these substances may escape from the cerebral ventricles to the systemic circulation and there change blood pressure, the doses used here would not be effective by peripheral administration [18, 13]. Therefore, it was considered that the role of the brain iso-renin–angiotensin system in the central regulation of blood pressure could be studied by this kind of experiment.

In normotensive rats intraventricular injection of saralasin and captopril caused no changes in blood pressure. These results are in close accordance with those of previous reports [8, 14], suggesting that angiotensin II formed in the brain is not directly related to the maintenance of normal blood pressure.

Injection of both saralasin and captopril into the cerebral ventricles caused a fall in the elevated blood pressure in two-kidney, one-clip Goldblatt hypertensive rats. These results confirm the previous findings of Mann et al. [8] with saralasin and show in addition that captopril is also effective in this type of hypertension. Thus locally formed angiotensin II in the central nervous system appears to be important in the maintenance of blood pressure in two-kidney, one-clip Goldblatt hypertensive rats. Since it is reported that circulating angiotensin II exerts its blood pressure-increasing effect by brain angiotensin II receptors [15], it is possible that this effect is blocked by saralasin.

Although a fall of blood pressure in spontaneously hypertensive rats after intraventricular injection of saralasin has been reported by several investigators [7, 8] the effects of captopril on
blood pressure have been controversial [13, 16] in this rat. In the present study injection of saralasin and captopril significantly decreased the blood pressure in spontaneously hypertensive rats. Since the levels of plasma renin activity were normal [17] and intravenous administration of angiotensin antagonist did not produce a fall in blood pressure of spontaneously hypertensive rats of this age [18], our results may provide confirmation that locally formed brain angiotensin II is responsible for the maintenance of high blood pressure in spontaneously hypertensive rats.

In DOC–salt hypertensive rats, contrary to our expectations, elevated blood pressure was further increased by central injection of saralasin and captopril. It is conceivable that brain angiotensin II exerts little effect on the maintenance of blood pressure in these rats, in which the peripheral plasma renin activity is suppressed. Our study, in which the intracerebral effects of these substances were examined, also demonstrated that both saralasin and captopril increased the blood pressure. The results for saralasin agree with those of Mann et al. [8] and it seems likely that the blood pressure increase by saralasin in these rats with low renin is due to its agonistic effects. Conversely, the hypertensive effects of captopril in these experiments are difficult to explain, since this substance has been shown to have no pressor effect by itself [19]. One possibility which explains such effects is that captopril may inhibit the degradation of bradykinin, since bradykinin is known to have pressor effects in the central nervous system [20].

In addition to the observation of blood pressure changes, plasma renin activity was measured after intraventricular injection of saralasin and captopril. Plasma renin activity has been reported to decrease when angiotensin II is injected into brain [4]. However, the mechanism still remains unknown. One possibility is an increase in plasma vasopressin concentration, since it is known that intraventricularly injected angiotensin II [21] stimulates vasopressin secretion and that vasopressin inhibits renin secretion [22]. Another possible factor is an increase in blood pressure, since angiotensin II increases blood pressure [2] and the blood pressure elevation is associated with suppression of renin secretion [23]. Although plasma vasopressin was not measured in this study, it is reported that it is elevated in experimental hypertensive rats such as renovascular hypertensive rats, DOC–salt hypertensive rats and spontaneously hypertensive rats [24, 25]. It is unacceptable therefore to consider that changes in plasma renin activity are related directly with blood pressure or vasopressin in these rats. Another mechanism may exist in the central regulation of plasma renin activity.

In conclusion, the present results suggest that the brain iso-renin–angiotensin system is important in the regulation of blood pressure in two-kidney, one-clip Goldblatt hypertensive rats and in spontaneously hypertensive rats. Conversely, the contribution of the brain iso-renin–angiotensin system appears to be of little influence on the maintenance of high blood pressure in DOC–salt hypertensive rats. It may also be suggested that the inter-relationships between the plasma renin–angiotensin system and the brain iso-renin–angiotensin system are complicated and variable in various forms of experimental hypertensive rats with different plasma renin profiles.

References


